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13. ABSTRACT

Over a series of years, the communication machinery was analyzed which serves to bring together intimately associated species in the sea (symbioses). The machinery may involve many types of stimuli, with visual and chemical perhaps the most important. The function of these signals has, to as great an extent as possible, been considered against the background of the ecology of the specific organisms themselves and the physical nature of the environment in which they may be found. During the most recent years, emphasis has been placed on the function and efficacy of chemical signals in the marine environment.

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ABSTRACT

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1967. D. Davenport, W. Hand and R. Forward. Short-term photic regulation in a dinoflagellate. *Biol. Bull.* 130: 150-165.
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The Experimental Analysis of Behavior in Symbioses

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Chuang-tzu and Hui-tzu had strolled onto the bridge over the Hao, when the former observed, "See how the minnows are darting about! That is the pleasure of fishes."

"You not being a fish yourself," said Hui-tzu, "how can you possibly know in what consists the pleasure of fishes?"

"And you not being I," retorted Chuang-tzu, "how can you know that I do not know?"

Chou of the Autumn Floods—
3rd Century B.C.

I. Introduction

Symbioses provide a world of interest for the student of animal behavior. An approach to them from the behavioral point of view reduces itself to an investigation of information exchange between two or more

biological systems of differing complexity. The complexity of the exchanging systems in turn determines the complexity of a symbiosis, both as a biological and sociological entity. It follows that the more complex the organization of the exchanging systems (or the "higher" their taxonomic position) the more difficult it is for us to investigate all the morphological, physiological, or behavioral machinery that controls an association.

In this concluding chapter we shall consider those specific behavioral acts or responses which serve the function of bringing symbiotic partners together or of maintaining them in association. Primary emphasis will be given to overt, quantitatively measurable activity, usually involving motion, and to the physiological mechanisms controlling such activity. No attempt will be made to be descriptively all-inclusive in our consideration of this machinery. A number of case histories familiar to the writer will be presented in an effort to indicate how the student of animal behavior may approach the investigation of symbioses and the experimental techniques he may use.

In the preceding material there is considerable information concerning the morphological, physiological, and biochemical changes that have occurred in the course of evolution of symbioses. However, in the great majority of such partnerships, there is less information concerning the natural economics of the partnership or its members. This information, which determines whether a particular symbiotic association will be called a commensal, mutualistic, or parasitic one, is so sparse in most cases that for the basic purpose of this chapter it will be to a great extent disregarded. In the examination of the behavioral machinery that effects and maintains an association, the economic relationship of the partners concerned is a secondary consideration, even though any benefit accruing to a partner must be what gives it a selective advantage over its free-living relatives and thus is probably the effective agent in the establishment of the specialized behavioral machinery.

In a stimulating series of recent reviews Sebeok (1969) reemphasizes the contention of the great language psychologist Bühler that "a systematic exchange of matter is inconceivable without an accompanying exchange of signs." In this chapter, emphasis will be placed on the exchange not of matter but of signals. We shall approach a symbiosis as a social entity made up of information-exchanging associates; the terms *commensal*, *parasitic*, *mutualistic* will be employed rarely and then only when we have objective evidence to support their use. In any such entity, particularly one in which the members are animals but also any in which the motile invasive stage of a plant or protistan is involved, specialized seeking behavior may be exhibited. If such behavior is exhibited by only one

partner, then from the behavioral point of view it is the active "seeker," while its associate (even though it may benefit by the association) is the passive member. If such behavior can be demonstrated in both partners, this is *prima facie* evidence of mutualism.

We return then, as always, to a problem in evolution. Having little quantifiable data on the economic or selective advantage accrued by any one species in a partnership, particularly in loose animal symbioses, we can only theorize about it. Yet it is quite clear that in many symbiotic species specific behavior has evolved that is *different* from that of their free-living relatives. These behavioral idiosyncracies can be precisely identified and the variation in stimulatory parameters eliciting them rigorously investigated.

As an example of the ways in which it may be possible to think about the course of evolution and the accompanying development of specialized behavior in symbioses, we may do well to consider initially that symbiosis best known to all, the partnership Man-Dog. If in the course of this consideration we are accused of anthropomorphism we have but to quote Chuang-tzu or the somewhat more recent *caution* of Dethier (1964): "Yet without a disciplined anthropomorphism inquiry into behavior is hobbled." In the Dog-Man association we are dealing with a system of extreme complexity in which both partners, being highly organized Mammalia, possess machinery of information exchange and storage far more efficient than do the members of the majority of partnerships we shall consider. This partnership arose during the Pleistocene or earlier, evolving from a situation in which Dog, a free-living species, moved into the orbit of the prehuman family and gained a selective advantage from the increased food supply, by scrounging tidbits from its more intelligent, predatory, and efficient host. The prehistory of this association can only be conjectured, but it is clear that by the ultimate acceptance of Dog as a working partner, Man changed the entire course of evolution of Dog. Dog is, as a result of genetic experiments carried out by his partner, enabled to undertake countless new activities which never appeared in nature prior to his domestication from the free-living condition. These are without doubt the result of the selection and recombination of genetic traits which were present in Dog's free-living ancestors. In effect, Dog has, as a result of specialized activity by his symbiotic associate, undergone all the first steps of behavioral speciation which appear to be maintained in purity as long as genetic isolation is maintained. A mastiff, boxer, or Doberman may be a better ranch guard than a Chihuahua. An Afghan is a more effective courser of game than a Pekinese. If isolation breaks down, then the genetically determined specialized behavioral characteristics established by Man may quickly disappear, much to the disgust

of the breeder. We have in fact almost no quantitative information concerning the relative importance in dog breeding of selection for external morphological differences, for new pathways in the central nervous system, or for new abilities in information-processing and storage. Yet few would doubt that Australian sheep dogs can be easily trained to do things which other strains, superficially similar in structure, take longer or fail altogether to learn. Similarly, we may have selected a strain in the bloodhound with a highly efficient stimulus-response chain for the processing of chemical information. There is every evidence that we have developed genetically determined behavioral polymorphism in the single symbiotic species, Dog.

What about the symbiotic future of the other partner, Man? In the course of ages of breeding experiments, Man, as the active agent in the evolution of Dog, has developed strains which have in turn conferred upon him selective advantages of importance in his own sociological evolution. Some dog strains hunt for Man, some herd for Man, some protect Man. Most strains clearly give Man an indefinable satisfaction; indeed Dog may take the place of Man's own offspring in a personalized, emotional, and highly mutualistic relationship. There seems little doubt that the association of Man with Dog has not only affected Man's physical evolution by increasing his chances of survival while he was in a primitive sociological state but has also in many undetermined ways affected his behavioral evolution. Any particular variation in either physical or sociological selective pressure on either partner of an association resulting from a change in the relationship of the partners must necessarily affect the course of evolution of the other, as well as of the symbiosis as a discrete biological entity. The symbiosis Man-Dog is not the same in the United Kingdom as it is in the United States. In Great Britain dogs are second-class citizens; the writer once observed a man in a restaurant feed a large steak to a boxer without eliciting so much as a raised eyebrow from either customers or management. In much of the United States dogs are now even kept off the beaches and out of parks, while in much of England they are welcome there. The association between Man and Dog, particularly during the developmental period of Man, has been shown by ages of empirical observation to be psychologically sound. What the long-term effects on Man or Dog may be of the increasing tendency to prevent small boys from taking their age-old companion to recreational areas cannot be determined. One must at least foresee a time when as a result of human crowding large dogs will cease to be a part of the social environment of man.

To conclude our digression, it may be observed that it is possible to

use symbioses in a very effective way in the prosecution of certain basic researches which have no direct bearing on the symbioses per se. This has certainly proven true of the symbiosis Man-Dog, for countless highly significant scientific advances have been made by Man using his partner Dog as an experimental subject in biological or medical research. Clearly, here, the association is parasitic.

Admittedly much of the above is conjectural. It is submitted merely as a design for the approach we shall now take in considering the experimental analysis of behavior in symbioses which are far more amenable to investigation than the complex, continuously varying, and somewhat unpredictable associations between Man and his domesticated partners.

II. An Experimental Case History

Although prior to 1930 a number of preliminary investigations were conducted on the chemical responses of the invasive stages of parasitic flatworms (Faust and Meloney, 1924; Barlow, 1925), it was not until 1930 that important experiments were performed which demonstrated the manner in which the behavior of an associated invertebrate may be precisely controlled by the receipt of specific signals from its host. John H. Welsh (1930) of Harvard University, demonstrated that the mite *Unionicola ypsilophorus* var. *haldemani* (Piers), which lives on the gills of the fresh-water clam *Anodonta cataracta* Say, moves away from a light source when it is under chemical stimulation by material from its host. Washing mites free of this chemical sign causes them to move toward the light source. The positive response to light of the clean mites can then be reversed by the introduction of material from the host to their vicinity. In later experiments (1931), Welsh worked with a series of three mite species inquiline with three distinct species of clams. He showed that in each mite species such reversal of the light response could be brought about by material from its host alone. This work is highly significant, for it not only represents the first demonstration of a high order of specificity in the transfer of chemical information from host to associate, but it also represents the first, and indeed the only, demonstration to date of the effect that a specific chemical sign from a host may have on the response of its associate to a different stimulus, in this case light. The demonstration of this phenomenon is significant enough, regardless of its importance in the control of the symbiosis concerned. However, it should perhaps be pointed out that although the response reversal is probably, as will be seen, of adaptive significance, the first demonstration of it was to an extent fortuitous. Yet the very fact that this dramatic

reversal of sign appears provides the clearest evidence of the value of the use of symbiotic associations as tools for the elucidation of fundamental physiological and behavioral phenomena per se. This point has been stressed before in a previous publication (Davenport, 1955). In any partnership in which specificity of response can be demonstrated, the student of behavior has an "organism-environment" relationship which, in one sense, is rather unique in that the experimenter can manipulate the primary source of stimuli (i.e., the host) for its associate in a way in which an environment which provides the main source of stimuli for a free-living organism can never be manipulated. This uniqueness is particularly true in partnerships in which the host releases into its environment specific chemical signals which may elicit responses from its associate *after* it has been removed from the system. This phenomenon, of course, gives the experimenter the opportunity of manipulating the source of stimuli not only in space but also in time. In the medium containing the chemical sign we are provided with a ready source of material with which the nature of the sign (thermal stability, solubility, etc.) may be investigated. Similarly, this manipulability confers upon the experimenter a unique opportunity to investigate stimulus-response chains of a high order of specificity. The frequently extreme specificity should, in turn, give us an opportunity to learn more about methods of transduction in sensors and the manner in which specific information is coded during transmission and integration. As will be seen below, there is every possibility that certain symbiotic invertebrates of a low level of nervous organization, such as actinians, may prove to be particularly useful for this purpose.

Although further work has not been carried out on the clam-mite association of Welsh, one can foresee an advance of fundamental importance when the reversal of the light response upon stimulation by a specific chemical is thoroughly understood. It may be mentioned in passing that the adaptive significance of the reversal of the light response seems obvious; larval mites which are free-living are dispersed while they are positive to light. When they fall under the influence of host factor (the timing of which may in part depend upon the stage of development), they become negative to light, descend, and are carried into the incurrent siphons of their hosts. A final question arises when one contemplates the reversal of sign in this partnership. This is the extent to which conditioning or learning may be concerned in it. It is possible that an answer may be gained in this case by a careful investigation of the relationships between responses to the two stimuli concerned and the relation of these responses to time.

III. The Identification of Mediating Machinery

The experiments of Welsh may be said to have established a pattern for the experimental analysis of behavior in symbioses. From them it became apparent that one must ask first of all what sorts of stimuli cause the active partner in an association to "seek" its host. They also established the importance of discerning what unique behavior, distinct from that of the seeker's free-living relatives, is exhibited by the active partner, the uniqueness lying in the adaptive significance of the behavior in terms of its importance in effecting or maintaining the symbiosis. The author's thinking about this machinery was in turn affected

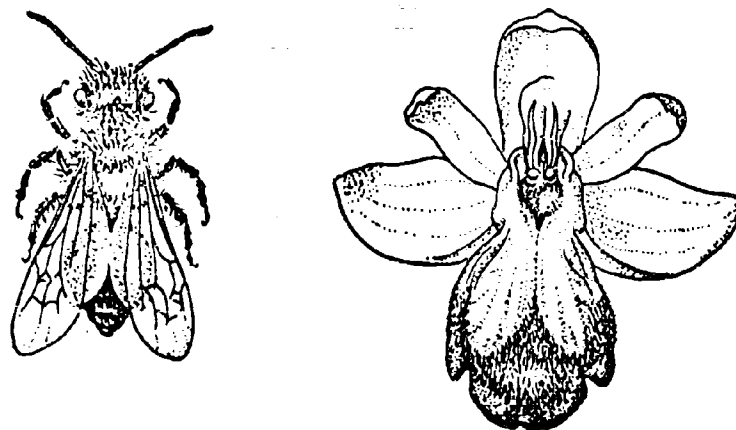


FIG. 1. The bee *Andrena trimmerana* and its partner in pseudocopulation, the orchid *Ophrys fusca*.

in the late 1940's by his becoming aware of the advances made by the ethologists (Lorenz, Tinbergen, Baerends, Thorpe, etc.), who demonstrated the evolutionary and ecological significance of a precise sort of information exchange between members of the same species. As is now well known, this involves the evolution of specialized structures or movements which serve to elicit specific adaptive responses from other members of the same species. These structures or movements are termed "releasers." Baerends (1956) pointed out the importance of this specific type of information exchange in symbioses using the now famous case of the relationship between a number of orchid species and certain Hymenoptera (Fig. 1) in which the hymenopterous insect cross-fertilizes the orchid during an attempt to copulate with the floret. Clearly here the almost

incredible resemblance of the libellum of the orchid floret to the hymenopterous insect is by the above definition an interspecific releasing mechanism; more recent work (Kullenberg, 1950, 1952) indicates that the orchid species involved in such symbioses produce chemical signs for the males of their associated hymenopterous species with a high order of specificity. These chemical signs appear to trigger the copulatory attempt. Whether such an association is a true mutualism has not been proved. Baerends says: "The orchids are especially fertilized by the insect as long as the female Hymenoptera have not left the pupae (the males usually hatch from a week to a fortnight earlier). During that time the males are likely to suffer from threshold lowering with regards to the sexual reactions. . . ." One might comment that Baerend's use of the word "suffer" indicates some benefit accrued by the male hymenopteron when he performs what the ethologists so delicately call the "consummatory act" with the orchid. Whether this benefit involves any selective advantage to the insect is unknown. That true releasers that have evolved to perform a particular interspecific function are of importance in many types of symbioses is clear; another case in point would be, of course, the evolution of such structures as honey guides in certain angiosperms such as the forget-me-not which in strongly reflecting ultraviolet light apparently provide a clear target for the nectar-seeking insect. In many cases, however, the releasers concerned in such mutualistic associations have not been identified.

We shall, however, have little occasion to use the term "releaser" in the discussion that follows. In almost all cases involving animals and many involving plants, the association, being a unilateral one (either commensal or parasitic), merely demands recognition by the seeker of some stimulus or pattern of stimuli from its host. Such a stimulus may be highly specific in its action without the implication that it has evolved as a special mechanism functioning to attract the seeker. For such specifically acting information we shall use the more general terms "sign stimulus" or "signal" whether or not the specificity of the total machinery concerned depends upon the nature of the signal itself (i.e., a characteristic of the sender) or upon particular physiological mechanisms intrinsic in the receiver. We have no great quantity of knowledge at hand concerning the nature of the signals which effect symbioses, but they may, of course, be classified in the same way as any other sort of sensory information received by an organism, i.e., they may act via photoreceptors, mechanoreceptors, chemoreceptors, etc.

IV. The Role of Photoreception

Symbiosis-adaptive responses to photic stimuli range all the way from simple kinetic or taxis responses to light, which (may) serve to increase the probability of host-finding, to perception of a specific image of the host by an associate. Welsh's above-described experiments provide an example of the former. Lees (1948), in his elegant analysis of the sensory physiology of the sheep tick *Ixodes*, has shown how unfed ticks respond to a sudden fall in light intensity by assuming the "questing" position in which the forelegs are extended rigidly to the front, giving the animal "an air of tense expectancy." This increases the chances of the parasite encountering its mammalian host. When the tick is gorged it is strongly photonegative and descends deeply into the vegetation. At the other end of the scale of importance of photic stimuli, recognition of discrete visual images or patterns must be a major factor in effecting certain partnerships. In the orchid-hymenopteron partnership, nature provides at least the strongest circumstantial evidence that the male insects recognize the libellum of the orchid which so much resembles the abdomen of the female. Were this not so it would be hard to imagine any mechanism whereby this astonishing resemblance had been selected for. Obviously, many other releasers which affect specialized behavior in the symbiotic associations between hymenoptera and the flowering plants they cross-fertilize must be visually recognized by the insect. In the field of marine symbioses, Arnold (1953) presents good evidence that part of the recognition of the host sea-cucumber *Holothuria tubulosa* Gmelin by its inquiline fish *Carapus acus* (Brunnich) depends upon the receipt of visual information. Fishes which live in association with sea urchins (Fig. 2) and with giant sea anemones (Fig. 3) certainly recognize their associates by visual means; the author and Dr. Kenneth Norris repeatedly observed the way fishes of the latter partnership orient themselves toward their host when prevented by a glass pane from receiving any other sort of information from it.

In another fascinating vertebrate-invertebrate association (see Chapter 7), that between a number of species of shrimps [*Paralimenes yucatanicus* (Ives) and *P. pedersoni* Chace; *Hippolytina grabhami* Gordon and *H. californica* Stimpson; *Stenopus hispidus* Oliver and *S. scutellatus* Rankin, etc.] and the numerous species of fish whose surfaces they clean, visual signs must be the most important method of information exchange in maintaining the relationship. The late Conrad Limbaugh of Scripps Institution of Oceanography, before his untimely death in a diving accident certainly one of the world's leading undersea naturalists, made a number of most interesting observations on cleaning symbioses in the

sea: these were later published (Limbaugh, 1961; Limbaugh *et al.*, 1961). These brilliantly colored shrimps generally have a well-established station, and frequently the station is in effect a symbiosis between the shrimp and a large sea-anemone [*P. pedersoni* with *Bartholomea annulata* (Lesueur)]. As has been so beautifully shown in a film taken by Harry Pederson on the Bahaman reefs, the crustacean "attracts the attention of a great variety of reef fishes by whipping its antennae and by swaying

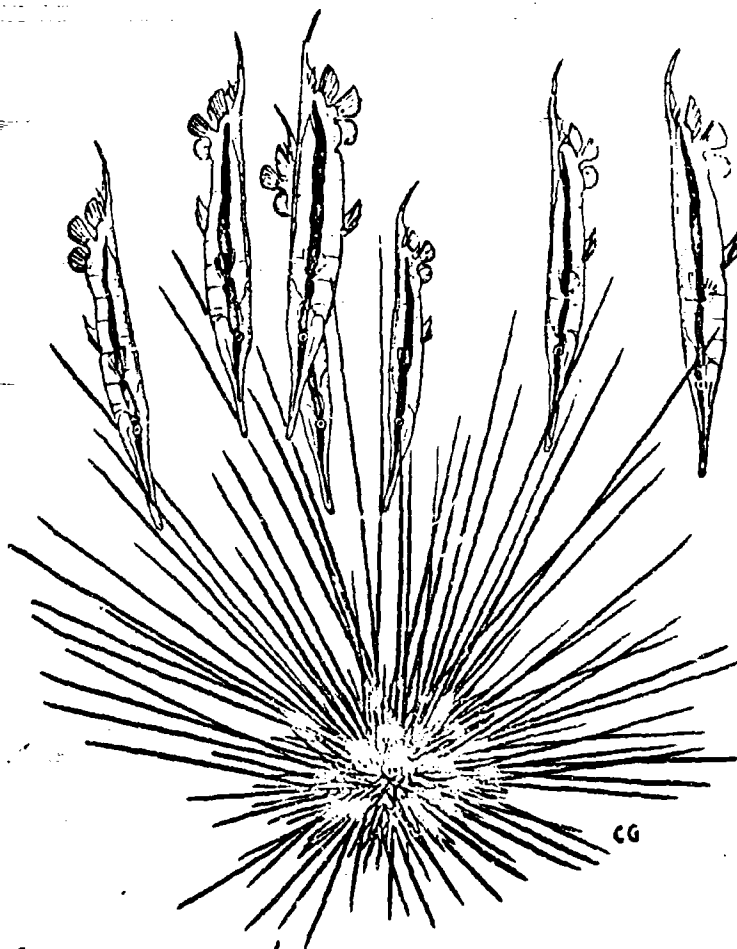


FIG. 2. *Aeoliscus strigatus* associated with the Long-Spined Sea-urchin.



FIG. 3. The pomacentrid fish *Amphiprion percula* with its anemone host *Stichodactyla* sp.

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back and forth from a prominent position on or near the anemone." This behavior must be of utmost significance in the maintenance of this particular symbiosis. The fish observe it and line up to be serviced, quite frequently pushing each other out of the way, for all the world like a group of impatient movie-goers in a long queue, in order to allow the shrimp to board them, enter their mouth, or dart beneath their operculum, which is obligingly held open. However, as has so often been the case, there has as yet been little effort to establish the exact nature of the signals which effect the response of fishes to the shrimp or vice versa. All subjective evidence points to a relationship between the shrimp's territoriality and its dancing, antenna-waving activity, but the importance of these factors has not been experimentally demonstrated. Certainly one of the most important sources of stimuli for the fish may be the color pattern of the shrimp. Experiments immediately come to mind in which modifications of the shrimp's activity and color could be made, both with the living animal or with models.

Two other facets of this symbiosis remain to be elucidated, both involving inhibition by the shrimp of certain activity of its partners. First of all one may wonder whether, as must be the case in many animals living with coelenterates, the shrimp in some way inhibits the nematocyst discharge of its anemone "office." Second, just what prevents the fishes, many of which are certainly predacious on crustacea, from ingesting the shrimp? That some cleaner-shrimps which have not as yet developed a fully effective inhibitory mechanism may be ingested by the fishes they clean has been demonstrated (Limbaugh *et al.*, 1961); an appreciable number of *H. californica* have been found in the stomachs of morays. How do successful species effect their protection? By color? Special behavior? Perhaps the antennal waving serves to inhibit predation and does not "attract" the fishes at all. Only experiments will answer these questions. It is fascinating to theorize about the course of evolution of this association, for it would seem almost certain that it could only have arisen between shrimps and fishes in which there was a continued danger of predation by the latter on the former; one might question whether any other relationship would have been intimate enough to permit the symbiosis to develop at all. During the course of evolution there may well have been a knife-edge balance between predation of fishes on these shrimps and their ability to inhibit the predation.

V. The Role of Mechanoreception

Even less is known about the role of the receipt of tactile information mediated by mechanoreceptors in symbioses. We shall in all probability find some larval symbiotic associates, the metamorphosis of which is

triggered only upon contact with a substrate of the correct *physical* nature. It will, as always, be difficult to be sure that the physical state of the substrate alone is responsible for metamorphosis and that a contact-chemical sense may not also be involved. In any such investigation a debt will be owed by the investigator to Douglas Wilson, who has so successfully demonstrated the importance of specific physical and chemical signals in eliciting the metamorphosis of a number of marine invertebrate larvae. Under any circumstance, perception of this sort must be important in symbioses. Passano and Pantin (1955) in working with the physiology of the actinian *Calliactis parasitica* (Couch) isolated from its host hermit crab but attached to a glass plate, noted that the anemone responds to jets of water by bending in the direction of the jet. The researches to be discussed below of Ross (1960), Ross and Sutton (1961a,b), and Davenport *et al.* (1961) have indicated the manner in which the physiology and behavior of the anemone are highly adapted to its particular symbiotic habit. Passano and Pantin hypothesized that the mechanical response is one that gives the anemone an advantage in feeding, when in actuality it would appear that the response is the first of a series which occur as the actinian recognizes and attaches to its host's shell. It would be interesting to determine whether this response to jets of water occur as frequently when the anemone is already attached to its host's shell as when it is isolated from it.

In unpublished work Gilpin-Brown showed that young *Nereis fucata* Savigny, the adults of which are inquiline in the shells inhabited by the hermit crab *Eupagurus bernhardus* (L.), make small tubes on the sea bottom. In aquaria he noted that if the substrate on which the tubes are placed was mechanically disturbed, these young nereids extended their heads from the tubes and made searching movements. Although Gilpin-Brown could not carry these experiments to the extent of observing colonization of the host, it would seem reasonable to assume that this response to mechanical disturbance may serve to increase the chance of the young worm making contact with a passing hermit crab. It would be most interesting to know more about the nature and specificity of the mechanical stimuli necessary to elicit this response.

VI. The Role of Chemoreception

Just as the receipt of specific chemical information is of great importance in the mediation of countless predator-prey and feeder-food-plant relationship, so it is of similar significance in mediating symbiotic associations. Chemical sign stimuli are perhaps maximally important in the maintenance of aquatic partnerships in general. For some years the author carried out (for the most part at the Friday Harbor Laboratories

of the University of Washington) a series of investigations of the role of chemical signals in marine symbioses, directing his attention largely to partnerships in which a polychaete annelid is the active, seeking member. In these experiments a number of types of apparatus have been used, reflecting the exigencies of particular problems to be solved and an increase in the efficiency in the design of apparatus to give more than one

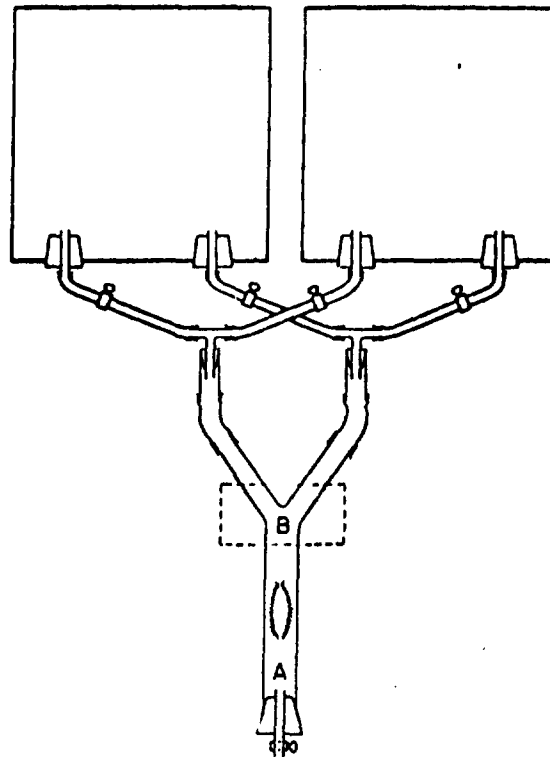


FIG. 4. Y-tube olfactometer.

sort of data at once. The first apparatus (Davenport, 1950) consisted of a simple Y-tube olfactometer (Fig. 4). Two identical aquaria were connected by cross connections to the arms of a glass Y. A slight bend in the stem of the Y at (A), a stopper with a stopcock at that point and four pinch clamps on the leads to the arms made it possible to fill the system with sea water, and by removing the stopper at (A), to insert a polychaete in the stem of the Y. The stopcock in the stopper at (A) was then opened slightly and leads to two of the arms opened, allowing material to come down from the aquaria to the point-of-choice (B). With this apparatus, if

no chemical sign from the host were in the system, a polychaete in position (A) would ordinarily remain quiescent, but when a lead to the aquarium containing the host was opened and the signal reached it, it would rapidly become activated, moving up to the point-of-choice (B) and entering an arm. Runs of this sort were scored + if the polychaete entered the arm containing sign, — if it entered the arm with unlabeled water. Presentation of chemical signal in one or the other arms of the Y could be randomized as a result of the cross-supply, thus controlling any possible variation in light or pressure at the point-of-choice (B).

This apparatus, although effective in our early experiments, had certain very definite shortcomings, among the worst of which was the tediousness of making enough runs using single animals to accrue adequate data for quantification and statistical analysis. In addition, one could measure with this apparatus only one sort of behavioral act, that is, the choice made by the worm when presented with streams of different nature. Studies could not be conducted on such phenomena as linear velocity or rate of change of direction under the effect of host factor. A further shortcoming was that the apparatus was not suited to test the behavior of organisms such as crabs which do not progress along the axis of their plane of bilateral symmetry. However, with the apparatus, initial studies were made which indicated the importance of proceeding with the investigation. These studies were conducted with members of a highly interesting genus of polynoid polychaetes, the genus *Arctonoë*, the members of which inhabit the ambulacral grooves of starfish, the surface of *Holothuria*, the mantle cavity of *Amphineura* and *Gastropoda*, the tubes of other polychaetes, etc. The recorded host habit of this genus can be seen in Table I. In our initial experiments we tested the responses of *Arctonoë fragilis* (Baird) and *A. pulchra* (Johnson) to sea water from aquaria containing their respective hosts, the starfish *Evasterias troschelii* Stimpson and the sea cucumber *Parastichopus californicus* (Stimpson). Both worms could discern and were clearly attracted to material coming from their host. Neither indicated any response whatever to water from the other's host, nor did either indicate any response to closely related nonhost stars or sea cucumbers.

The apparatus is reasonably well suited to gain information about the nature of chemical signs, except for the tediousness of single-run operations. It was first found (Davenport and Hickok, 1951) that aquarium water from which a host *Evasterias* had been removed 24 hours before no longer possessed any attraction for its polychaete associate. If water from an aquarium containing a starfish effected strong responses, rather forcible removal of the starfish from the wall of the aquarium, which left torn-off tube feet on the aquarium wall, would immediately

TABLE I
REPORTED HOSTS OF THE COMMENSAL POLYPOID GENUS *Archimedes*

<i>A. fragilis</i>	
Echinodermata	
Asteroidae:	<i>Evasterias buschelti</i> Stimpson <i>Leptasterias nequalis</i> <i>Leptasterias hexactis</i> (Stimpson) <i>Orthasterias kachleri</i> (de Lariol) (= <i>columbiana</i> Verrill) <i>Pisaster ochraceus</i> (Brandt) <i>Solaster dawsoni</i> Verrill <i>Stylasterias furrei</i> (de Lariol)
<i>A. pulchra</i>	
Echinodermata	
Asteroidae:	<i>Luidia foliolata</i> Grube <i>Pteraster tessellatus</i> Ives <i>Solaster stimpsoni</i> Verrill
Holothuridae:	<i>Parastichopus californicus</i> (Stimpson)
Echinoidea:	"Sea urchin" (vide Chamberlin)
Mollusca	
Amphineura:	<i>Cryptochiton stelleri</i> Middendorf
Gastropoda:	<i>Megathura crenulata</i> (Sowerby)
Annelida	
Polychaeta:	<i>Loimia montagui</i> (Grube)
<i>A. villata</i>	
Echinodermata	
Asteroidae:	<i>Dermapteraster intricata</i> (Grube) <i>Pteraster tessellatus</i> Ives <i>Crossaster papposus</i> (Linné) <i>Henricia leviurcula</i> (Stimpson) <i>Luidia foliolata</i> Grube <i>Solaster stimpsoni</i> Verrill <i>S. dawsoni</i> Verrill <i>S. endeca</i> (Linné) <i>Asterias amurensis</i> Lütken
Mollusca	
Amphineura:	<i>Cryptochiton stelleri</i> Middendorf
Gastropoda:	<i>Diodora aspera</i> Eschscholtz <i>Puncturella cucullata</i> Gould <i>Puncturella multistriata</i> Dall <i>Acmaea mitra</i> Eschscholtz <i>Cidarina cidaris</i> Adams <i>Fusitriton oregonense</i> (Redfield) <i>Haliotis kamschatkana</i> Jonas <i>Patelloida</i> sp.
Annelida	
Polychaeta:	<i>Amphitrite robusta</i> Johnson <i>Thalopus crispus</i> Johnson

* After Pettibone (1955).

abolish the responses. If a host star was suspended in a bolting-silk bag in an aquarium and then carefully removed, the water in the aquarium retained its attraction for a period of between 15 and 30 minutes. If the host starfish was suspended in an aquarium overnight in a dialyzing bag, the water outside the dialyzing bag effected no attraction. Splitting the bag with a razor blade immediately brought about positive responses. Finally, a number of types of preparations were tested to discern attractability of parts of hosts, i.e., washed, eviscerated integument of *Parastichopus* and *Evasterias*, ground-up preparations of *Evasterias*, and washed viscera of *Parastichopus*. None of these elicited positive responses and, some, indeed, appeared to repel the polychaete associates.

There is obviously considerable variability in the ability of associated annelids at least to recognize their hosts by chemoreception from a distance. Concurrently, with the above experiments, tests were run on the well-known associated polynoid, *Halosydna brevisetosa* Kinberg, which had been collected from the tubes of the large terebellid *Amphitrite robusta* (Johnson). Even when as many as 19 large *Amphitrite* were placed in an aerated test aquarium for 12 hours, the water surrounding them had no attraction whatever for *Halosydna*; distribution in the arms of the Y was random. It was noted, however, that if this large commensal came in contact with the delicate tentacle of its host terebellid, it would move directly along the tentacle until it came in contact with the body of the host, whereupon it would wrap itself around the host.

These observations led to a number of experiments later conducted at the Marine Biological Laboratory, Plymouth (Davenport, 1953a). In the case of the scale worm *Acholoë astericola* (Delle Chiaje) associated with the starfish *Astropecten irregularis* (Pennant), no response whatever could be demonstrated when a host star was brought within a few millimeters of the worm's head, but if contact was made, the response of the polynoid was immediate and characteristic. If a tube foot was presented to the head of the polynoid it would cling to the tube foot and wrap its body around it immediately. This wrapping response made possible a number of studies in specificity which were of some interest. Table II indicates the results of testing this polynoid against a number of starfishes in Plymouth waters. As can be seen from the table, worms responded consistently both to the host *Astropecten* and to the starfish *Luidia ciliaris* (Phillippi). It is interesting to note that the worm is symbiotic with *Luidia* in southern Europe, but has not been recorded with it in Plymouth Sound. The data indicate that *Acholoë* responds to some extent to all members of the asteriod order Phanerozonia available at Plymouth (*Astropecten*, *Luidia*, *Porania* but not *Solaster*), to certain spinulose stars (*Asterina*, *Palmipes*, *Henricia*) but not ordinarily to

TABLE II
RESPONSE SPECIFICITIES TO EXCISED TUBE FEET OF THE COMMENSAL POLYPOID *Acholoë astericola*
COLLECTED FROM *Astropecten irregularis*^a

Worm	<i>Astropecten irregularis</i>	<i>Luidia ciliaris</i>	<i>Palmipes membra-naceus</i>	<i>Henricia sanguinolenta</i>	<i>Asterina gibbosa</i>	<i>Porania pulchellus</i>	<i>Marthasterias glacialis</i>	<i>Asterias rubens</i>	<i>Solaster papposus</i>	<i>Stichasterias rosea</i>
1	+	+	(+)	(+)	(+)	(+)	0	0	0	0
2	+	(+)	(+)	(+)	(+)	(+)	0	0	0	0
3	+	+	(+)	(+)	(+)	(+)	(+)	+	(+)	0
4	+	+	(+)	(+)	(+)	(+)	0	0	0	0
5	+	+	(+)	(+)	(+)	0	0	0	0	0
6	+	(+)	(+)	(+)	(+)	0	0	0	0	0

^a KEY: Characteristic response, +; no response, 0; partial response, (+).

^b The table is arranged according to intensity of response; in the experiments the starfish whose tube foot was presented to the team of worms was randomly selected.

^c Small size of tube feet necessitated presentation of tip of arm of starfish.

forcipulate stars (*Asterias*, *Marthasterias*, *Stichasterella*). This would appear to indicate some possibility of biochemical similarity between members of each group. In addition, it was possible with the contact technique to determine something about the source and nature of the attractant for *Acholoë*. The situation does not appear to be exactly similar to that in *Arctonoe*, although the testing techniques were, of course, not the same. In *Acholoë* any excised material from the host appeared to elicit the characteristic contact response, and there was evidence that the stomach of the host was the richest source of chemical signal. Isolated tissue appeared to retain its attraction as long as it remained fresh and alive. Chilling the tissue did not appear to alter its attraction, but temperatures above 45°C abolished it. The signal could be adsorbed on cotton merely by placing bits of cotton in the stomach of the host. If such bits of cotton were placed in a moist chamber and tested some 5 minutes after removal from the stomach, they still elicited positive responses, but by the end of 15 minutes they had lost their effect. There is clear indication here of the instability of the signal in air.

The admittedly somewhat subjective method of testing responses by direct contact made it possible, nevertheless, to carry out at Plymouth some extremely interesting studies on specificity, which are believed to have some significance from the point of view of the evolution of new host habits (Davenport, 1953b). A particularly interesting ecological situation exists in the large masses of intertwined tubes of *Chaetopterus variopedatus* (Renier) which may be dredged from the Eddystone grounds. In between inhabited and within abandoned tubes there frequently occurs the handsome strawberry-colored terebellid *Polymnia nebulosa* (Montagu). About 30% of these are accompanied by the symbiotic *Polynoë scolopendrina* Savigny. Tests of the responses of this worm indicated a much more precise specificity than that shown in the above-described tests with *Acholoë*. A *Polynoë* gave no responses whatever to any worm not a member of the family of its host. Tested against a spectrum of polychaetes in the family Terebellidae the polynoid gave the responses seen in Table III(A). It is to be noted that consistent responses were given to the host alone, but reduced responses were given to two members of the genus *Amphitrite* while no responses whatever were given to *Terebella lapidaria* L. and *Polycirrus caliendrum* Claparède. However, one of the most interesting and, we believe, significant facts in these studies emerged when this polynoid was tested against two forms which have been reported as alternate hosts, *Lanice conchilega* (Pallas) (which is again a terebellid) and *Lysidice ninetta* Audouin and Milne-Edwards (which is not a terebellid, but a eunicid). Table III(B) indicates the results. As can be seen, the polynoid responded strongly to an alternate host in Plymouth waters

TABLE III
 RESPONSES OF *Polynoe scolopendrina*^a

(A)				
Worm	<i>Amphitrite edwardsi</i>	<i>Terebella lapidaria</i>	<i>Polycirrus calidrum</i>	Host control
1	0	0	0	+
2	0	0	0	0
3	0	0	0	+
4	0	0	0	+
5	0	0	0	+
6	0	0	0	(+)
7	0	0	0	+
8	0	0	0	+
Worm	<i>Amphitrite johnstoni</i>	<i>Amphitrite gracilis</i>	Host control	
9	0	0	+	
10	0	(+)	+	
11	0	+	+	
12	0	+	+	
13	+	0	+	
14	0	0	+	
15	0	(+)	+	
16	(+)	(+)	+	
(B)				
Worm	<i>Lanice conchilega</i> (Terebellidae)	<i>Lysidice ninetta</i> (Eunicidae)	Host control	
1	0	(+)	+	
2	0	(+)	+	
3	0	+	+	
4	0	+	+	
5	0	+	+	
6	0	0	+	
7	0	+	+	
8	0	+	+	
Worm	<i>L. conchilega</i> ^b	Host control		
9	0	+		
10	0	+		
11	0	+		
12	0	0		
13	0	+		
14	0	+		
15	0	+		

^a KEY: As in Table II.^b The tests against *Lanice* were repeated with fresh material.

which belongs to a totally different family of polychaetes than its host *Polynnia*. It did not respond to *Lanice* with which it has been described from deep water on the French coast, in spite of the fact that *Lanice* is a terebellid. Some rather similar information was obtained with tests on the polynoid *Harmothoe spinifera* (Ehlers), which lives in association with a terebellid *Amphitrite gracilis* Grube and has also been described from the terebellid *Polycirrus caliendrum*. A population of this polynoid collected from *Amphitrite gracilis*, when tested against the host, two other members of the genus *Amphitrite* and *Polycirrus caliendrum*, gave uniformly strong responses to the host and to *Polycirrus* but reduced responses to two terebellids in the same genus as the host. This experiment and the above-described tests with *Polynnia* yielded some most interesting information, for the same phenomenon appeared in both: Some symbiotes have populations living with unrelated hosts, let us say *A* and *B*. A symbiote collected from *A* may, however, give equally strong responses to *B*, in spite of the fact that *B* is unrelated to *A*. Furthermore, said symbiotes may give much stronger responses to the alternate host *B*, than to *A'* or *A''*, nonhosts in the same genus as *A*. The significance of this sort of specificity in the establishment of new associations has been discussed elsewhere (Davenport, 1955). Put in the simplest way it would seem entirely possible that the fortuitous production by some nonhost animal of a metabolite similar to the chemical signal of a host may very well lead to an establishment of an association with it, all other factors being favorable.

However, drawing conclusions from an analysis of response specificity is not easy. Table IV gives some indication of the situation when one tests the responses of the polynoid *Harmothoe lunuleta* (Delle Chiaje) against a broad spectrum of alternate hosts. Three populations of *Harmothoe* were tested against this spectrum, one from the brittle star *Acrocnida brachiata* (Montagu), one from the holothurian *Leptosynapta inhaerens* (O. F. Müller), and one from the terebellid polychaete *Amphitrite johnstoni* Malmgren. It is interesting to note that those worms collected from the brittle star responded with strong positives to their own host, to the alternate host *Leptosynapta*, but to no other alternate hosts; the population collected from *Leptosynapta*, although admittedly small, responded to their host only, curiously enough not demonstrating the double specificity of their relatives that inhabited the brittle star. Polynoids collected from *Amphitrite* responded with fuller intensity to their host and gave occasional partial responses to *Leptosynapta* and a number of alternate annelid hosts. The significance of this type of response specificity remains unexplained.

Not long after the completion of the above studies in Plymouth, the

TABLE IV
RESPONSES OF THE POLYSOME *Haemaphysalis lunulata* TO ALTERNATE HOSTS^a

Worm	<i>Leptosynapta</i> infect.	<i>Amphitrite</i> <i>johnstoni</i> tentacle	<i>A. edwardsi</i> tentacle	<i>Polysinus</i> (sp?)	<i>Arenicola</i> <i>marina</i>	<i>Lance</i> tentacle	<i>Phoron</i> <i>lovenae</i> clong	<i>Arenicola</i> <i>lunulata</i>
Experiment No. 1. Commensals (3-12 mm long) with <i>Arenicola</i>								
1	0	0	—	—	—	—	—	(+)
2	+	0	—	—	—	—	—	+
3	+	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	(+)
5	(+)	0	0	0	0	0	0	+
6	(+)	0	0	0	0	0	0	+
7	(+)	0	0	0	0	0	0	+
8	+	0	0	0	0	0	0	+
9	0	0	0	0	0	0	0	0
10	+	0	0	0	0	0	0	+
11	+	0	0	0	0	0	0	+
12	+	0	0	0	0	0	0	+
Experiment No. 2. Commensals (29-31 mm long) with <i>Leptosynapta</i>								
1 ^b	+	0	—	—	—	—	—	0
2	+	0	0	(+)	0	0	0	0
3	+	0	0	0	0	0	0	0
Experiment No. 3. Commensals (16-22 mm long) with <i>Amphitrite johnstoni</i>								
1 ^b	0	+	—	—	—	—	—	0
2	+	+	0	(+)	(+)	0	0	0
3	0	+	0	0	(+)	(+)	+	0
4	(+)	0	0	(+)	(+)	0	(+)	0
5	0	+	0	0	0	0	0	0
6	(+)	+	0	0	0	0	0	0

^a Key: As in Table II.

^b First sample collected; untested alternate hosts not available.

never became obvious for an olfactometer that would give more than just one sort of information. It became apparent that it was important to determine rather more precisely what host factor makes an associated animal eat. It was clearly necessary to quantify the effects of host factor on linear velocity and rate of change of direction, and to determine the effects of gradients on such activity. Furthermore, it had frequently been necessary to compare response specificities in different populations of a single symbiotic species, some of which could easily be collected in large

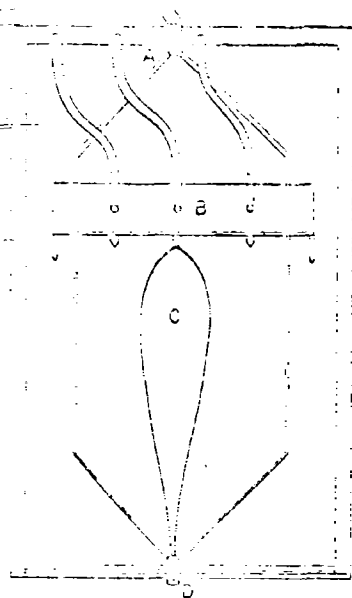


Fig. 5. Trough olfactometer.

numbers and others of which were extremely rare—so rare indeed that one might only have one or two individuals with which to experiment. In such a situation there was always difficulty in obtaining enough quantitative data from members of the rare population for statistical analysis. These problems were solved to a great extent by the development of the apparatus shown in Fig. 5 (Davenport *et al.*, 1960). This was based on an olfactometer developed by Varley and Edwards (1953) for studying host-finding behavior in parasitic insects. The adaptation consists of a hexagonal trough in transparent Plexiglas 17" by 8 $\frac{3}{4}$ " by $\frac{1}{8}$ ". The trough was made by cutting a hole of the above dimensions in a Plexiglas sheet and cementing it to a whole sheet. Sea water was introduced through a

tube (A) at one end of the trough. At (B), three streams of sea water were introduced from drip tubes set in a plastic bridge across the width of the trough. Noncirculating sea water was supplied to the three tubes from three beaker beakers which were maintained in a large aquarium water bath at the temperature of the laboratory sea water system. The flow from the drip tubes could be regulated by screw clamps so that rate of flow in the three resulting streams in the trough could be equalized. One of the three beakers was used as a test aquarium into which fluorescein (10⁻⁵ by weight) and hosts were placed. Controls indicated that the inert dye fluorescein has no effect on the behavior of experimental animals, and it made the central stream and its limits carrying effective agents clearly discernible to the observer. In the very slow current moving from one end of the trough to the other this labeled central stream formed a spindle-shaped area (the "critical" area-C) from the central drip tube to the outlet (D), limited on each side by streams of smoothly flowing unlabeled water moving at the same rate. The entire trough was mounted on a wooden frame which had a slot in it so that a sheet of ground Plexiglas could be introduced directly below the trough. The track or pathway made by an experimental animal could be traced from beneath, on this removable ground sheet, which at the end of a test run could be taken out of the apparatus without disturbance and the track permanently recorded from it on tracing paper. During the recording of an experimental track on the ground sheet, small marks were made perpendicular to the track at the sound intervals of an audible timer. A wheeled distance indicator or planimeter of the type used to measure distances on maps was used in determining distances traveled from the permanent records. This apparatus proved to be remarkably useful, although an automatic method of recording pathways and time intervals would be a worthwhile addition to it.

Figure 6 shows the kinds of pathways described in this apparatus by the hesionid polychaete *Podarke pagettensis* Johnson under three sets of conditions. In Southern California this polychaete has a free-living population, and a population associated with the starfish *Patiria miniata* Brandt; the two are morphologically indistinguishable. The left-hand track is that of a member of the free-living population with the central area containing fluorescein and water from an aquarium containing *Patiria*. The center track is that of an associated worm running against a central stream containing fluorescein only. The right-hand track is that of an associated *Podarke* running against a central stream containing fluorescein and water from an aquarium containing *Patiria*. In this figure the time signal is not indicated. One can immediately observe, however, that data are now at hand to give the answers to a number of questions.

Thus, one can examine the tracks to see whether the behavior of the symbiotic animal when in host factor is different than its behavior out of it, and if so, how. One can observe changes in behavior, if any, as the animal moves across a steep interface or gradient of chemical signal. One can then, if he discerns differences induced by the chemical signal, analyze these differences to determine whether or not they can be categorized as kinetic (nondirectional) or taxis (directional) responses. Analysis of pathways indicated a number of things about the behavior of the symbiotic *Podarke*. First the worm speeds up markedly under the influence of host

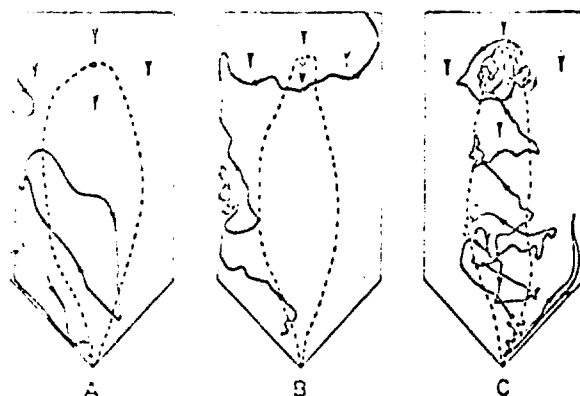


FIG. 6. Tracks of *Podarke pugettensis* in the trough olfactometer. A, a free-living worm, central area containing host-labeled water and fluorescein. B, commensal worm, fluorescein only. C, commensal worm, host-labeled water with fluorescein.

factor. In Fig. 7 where distance traveled is plotted against time intervals, we see that the increase in linear velocity occurring when the animal first encounters chemical signal rapidly drops off and returns to a norm, probably as the result of sensory adaptation. Pathways were analyzed to determine whether there was any variation in the rate of change of direction when the *Podarke* encountered sign. When one used the admittedly somewhat subjective method of Ulyott (1956) to determine the number of changes of direction per centimeter of track or per unit time and when one analyzed these data statistically, it emerged that the presence of signal induced no significant change in the rate of change of direction. As Fig.

6, which is a characteristic positive, indicates, the associated *Podarke* appear to be physiologically trapped in a host-labeled stream. They "climb up" the current, as they do whether or not signal is in it. Yet where they cross the interface between host-labeled and non-host-labeled water, they turn toward the source far more frequently than away from the source whether they are moving out of the host-labeled stream or into

it. The sense organs of the *Podarke* are therefore obviously being asymmetrically (directionally) affected. It would appear, then, that in symbiotic *Podarke pugettensis* a positive tropotaxis is probably the most important behavioral mechanism involved in host finding. There is no apparent klinokinetic response (change in the rate of random change of direction) caused by chemical signal. Chemokinetic responses are therefore of less importance, although there is clear evidence that a "direct" orthokinetic response (in which linear velocity is directly proportional to intensity of stimulation) occurs when the worm first encounters host-labeled water.

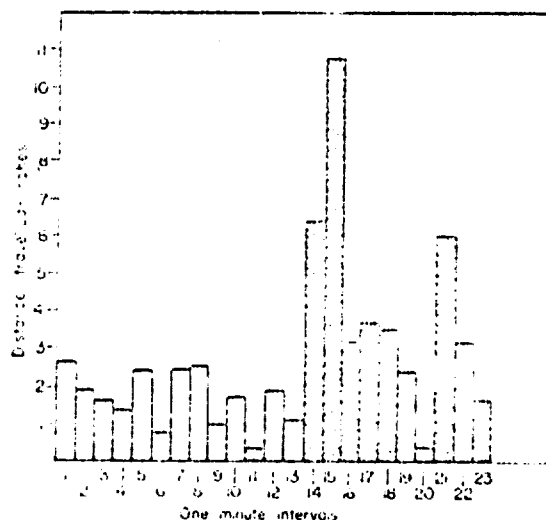


FIG. 7. Orthokinetic response of a commensal *Podarke pugettensis* when encountering host-labeled water. Unshaded bars indicate distance traveled per unit time when outside the labeled streams, shaded when in labeled stream.

The development of this apparatus also provided an immediate opportunity to analyze the behavior of some well-known associates which do not move along the axis of their plane of symmetry. The symbiosis between crabs of the family Pinnotheridae and a number of marine invertebrates has long attracted the attention of marine ecologists. Investigation of the behavior of the crab *Pinnixa chaetoptera* Stimpson, relative to its host *Urolopterus pinnatifidus* Cuvier proved to give an insight. This animal shows no reaction to current and moves in a perfectly random way in the absence of a chemical signal in the stream.

Shown in Figure 8, Figure 8a shows that the animal is "physiologically attracted" to a host-labeled central stream but obviously the attraction is more deviant than in a stream which is unlabeled. One may explain

whether this deviousness is affected by encountering interfaces, but Fig. 8C shows that the devious pathway is retained even when the *entire* trough is lined with signal. This deviousness is highly interesting, because statistical analysis of the data indicates that frequency of turning is proportional to intensity of chemical stimulus, as dependent upon the number of *Chaetopterus* placed in the test aquarium. This is a typical "direct" klinokinetic response. Under these experimental conditions, in spite of the fact that frequency of turning increases with rise in intensity of stimulus, the plotted net displacement of a large number of animals was *random in direction*; they were not, by the klinokinetic mechanism,

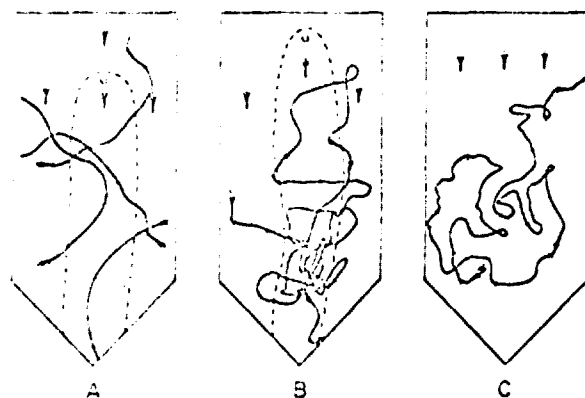


FIG. 8. Tracks of *Pinnixa chaetoptera*. A, four tracks made by a single crab, central stream carrying fluorescein only. B, same crab run immediately against host-labeled water with fluorescein. C, track of a crab in trough entirely filled with host-labeled water.

caused to move up the gradient in the central stream toward the source. That the behavior of these crabs is also mediated by a chemotactic (directional) response is indicated by the fact that careful statistical analysis of crab track pathways shows that they can discern the difference at the interface between the central host-labeled stream and pure sea water, more frequently than not, turning back into it when they reach the interface.

It is obvious that this apparatus also provides an extremely effective medium for the investigation of specificities and the nature of chemical signals. *Paralell* to *Chaetopterus* were shown to respond readily to their alternate host *Amphitrite ornata* Verrill, but paid no attention to water containing *Nereis* or *Arenicola*, both nonhost polychaetes. It was found possible to elicit responses to *Chaetopterus*-water as much as 24 hours after the removal of the host worms. Raising the temperature of host-

labeled water to 50. For 20 minutes eliminated all traces of activity. When hosts were suspended in a dialyzing bag, the water outside the bag gave no indication of activity. In a number of other tests, including treatment with trypsin, information was obtained which indicated that the chemical signal was probably a relatively time-stable protein.

VII. Symbiosis: A Tool for the Investigation of Basic Physiological and Behavioral Phenomena

The above description begins to indicate how, with the development of effective apparatus, symbioses may provide a particularly effective tool for the investigation of behavior *per se*. As has been previously stressed, this depends, as much as anything else, on the relative ease with which the primary source of stimuli for the associated "seeker" may be manipulated. Largely as a result of these experiments, the writer has become extremely interested in one phenomenon exhibited by the crab, namely, the direct klinokinetic response. This phenomenon, in which rate of change of direction varies directly with intensity of stimulation, has long attracted the interest of students of physiology and behavior. It should always be kept in mind that, by definition, the direction of klinokinetic turning is random. If this is the case, what function does the behavior have in "target finding?" The phenomenon undoubtedly occurs in many phyla of animals, but has only rarely been demonstrated when the possibility of directional stimulation has been entirely eliminated. This appears to have been done by Dethier (1957) in his significant studies of the effects of feeding different concentrations of sucrose to the fly *Phormia*. The physiological machinery effecting this response may be established in the genetic code of certain species and not others. If this is the case, we must perforce direct our attention to the question of how this machinery imparts a selective advantage to the species that has it. Personal communication with a number of workers who have been interested in such responses (Fraenkel, Wigglesworth, Dethier, J. S. Kennedy) indicates that there has been no experimental demonstration of the efficacy of a direct klinokinesis in bringing about aggregation *at or near* the point of highest concentration or intensity of stimulus. The above-described experiments with the pinnixids, in which the rate of random turning increased with the concentration of host factor and in which, nevertheless, crabs failed to move up the gradient that existed in the central host-labeled stream, appear to indicate that under these conditions, at least, the direct klinokinetic response acting alone did not cause the crab to come closer to the stimulus source. One might suppose that, in effect, any mechanism which reduces the length of straight runs

in a constant medium, organism moving at a constant linear velocity will act as effective a delaying or trapping mechanism as an inverse orthokinesis in which linear velocity decreases with a rise in intensity of stimulation. However, it must not be forgotten that, in a gradient or no, by definition the turning in the klinokinetic phenomenon is random in direction, which means that an organism "under klinokinetic drive" *alone* has an equal chance of turning up or down a gradient. Every time it turns down the gradient the length of its straight run increases, and at the next turn it has again a chance of turning up or down. Among workers there is some agreement that aggregation of directly klinokinetic animals will occur near the point of highest intensity only if their sensory adaptation is slow and that rapidly adapting animals would under such conditions tend to aggregate away from the highest intensity. But there is no clear experimental demonstration that a particular rate of sensory adaptation is necessary to bring about aggregation by direct klinokinesis, the theory of Ulfyott (1936) notwithstanding. This is so, at least, because no experiments have been performed in which the possibility of directional stimulation in the gradient has been eliminated. In organisms with symmetrical sense organs, there is always the possibility that one sense organ will be stimulated at a different level than the other when the animal is in a gradient, and in organisms with single sense organs, the phenomenon defined by Fraenkel and Crick (1937) as a *kemonaxis* (repetitive sampling of intensities of stimulation in time) may actually be the effective aggregating agent. In the above experiments with pondichids, when the trough was entirely filled with test factor, or a high concentration, the animals made tighter turns than when it was filled with a low concentration, yet in a host-labeled central stream which clearly had a gradient from source to exit, the animals failed to move consistently either toward or away from the source. It is, of course, possible that the gradient in the central stream was not steep enough, for it was clear that the animals responded in a directional way (maxitaxis) only when, at the margins of the stream where the gradient was steep.

Under the particular experimental conditions of investigating these animals it was not possible to indicate aggregation near the source of stimulation even when a direct klinokinesis and positive chemotaxis were clearly present in the experimental animals. These results are not to be understood. One may be sure that in any relatively automatically controlled locomotory, prey, or food-finding behavior, rate of sensory adaptation, or the "weaker," steepness of gradient of stimulation, and the physical nature of the medium transporting the chemical sign from its source may play a vital part in the total target-finding machinery. Because of the

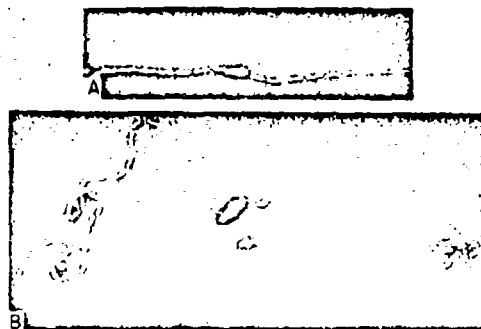
extreme difficulty of controlling and isolating all these factors and in particular of eliminating the possibility of taxis responses affecting results obtained in a study on klinokinesis, some theoretical computer studies have recently been undertaken, the results of which are as yet inconclusive and therefore still unpublished. Theoretical animals are traced on a grid representing a gradient from high to low levels of stimulation. From any single point on this grid a theoretical animal has, every time it moves, a certain probability of making a forward move of fixed length, a certain probability of making a 90° turn to the left of similar length, a certain probability of making a 90° turn to the right of similar length and 0 probability of a backward move. Preliminary studies of this sort in which a direct klinokinesis was simulated (with taxis eliminated) by keeping the probability of right or left turns equal but by varying the probability of a forward jump from high probability at the end of low stimulation on the grid to low probability at the end of high stimulation on the grid indicate that a large number of animals thrown onto the grid show after the passage of 100 jumps a net displacement which is totally random in direction. This approach has also enabled us to demonstrate that statistical aggregation occurs, as one might expect, at the high-stimulation end of the grid if we simulate the effects of the taxis response alone by keeping the probability of forward motion constant but making the probability of turns up the gradient higher than down the gradient. It should be at least theoretically possible to find out whether a particular relationship between these behavioral phenomena may increase the chances of reaching the target: the effects of sensory adaptation can also be simulated. It would seem reasonable to assume that the tropotaxis response as defined by Fraenkel and Gunn, in which symmetrically arranged sense organs are asymmetrically stimulated, is a relatively efficient target-finding mechanism. But it would also appear to be possible, although it has not as yet been demonstrated, that an animal behaving in this way and at the same time possessing a stimulus-response machinery which causes it to alter its linear velocity and rate of change of direction as it moves into a region of more intense stimulation *may* have a more efficient target-finding mechanism than an animal which possesses the taxis response alone. The probability that no such organism as the latter exists in nature gives evidence of the adaptive significance of kinesis, but this should be demonstrable on mathematical grounds.

This investigation of the role of klinokinesis in host-finding in symbioses triggered the development of new apparatus designed to study klinokinetic phenomena and other types of behavior in the invasive stages of certain associated animals which are too small to observe in

apparently none of the above described troughs. The miracidium of the adult *Schistosoma mansoni* (pamphili) was seen doing a "devil dance" (Robert and Nielsen, 1924) when in the presence of material from its host snail *Mastomys natalensis* (Savi); the dance was a whirling activity of great speed and deviousness. While the writer was working with Dr. C. A. Wright of the Department of Zoology of the British Museum in an effort to record pathways described by this miracidium under varying conditions, it became apparent that special apparatus would have to be developed which would enable one to record such rapid and sometimes extremely devious pathways. Fortunately, there was available in London a flyingspot particle-resolver of the type which had been developed by Professor J. Z. Young of the University of London to count the number of labeled neurons in fixed tissues, etc. The apparatus essentially allows one to observe on a visual screen and at the same time photograph a televised image of a fixed or moving microscopic object. In addition to the advantages described by its original designers (Roberts and Young, 1922), the apparatus has a number of others which make it particularly useful in the study of the behavior of motile microorganisms (Davenport *et al.*, 1952). The difficulty encountered in making a manual record with the aid of a camera lucida of the movements of a microorganism which describes a devious pathway while moving swiftly (such as a miracidium or protozoan) results simply from the inability of the observer to follow such movements. One can, of course, record the movements with movie film, but this requires much film and hours of processing, tracing, and measuring if one wishes to obtain quantitative data on the behavior of the individual organism. One could also make direct short-duration time exposures of the organism under dark-field stroboscopic illumination. The flyingspot system minimizes the possible effects of directional illumination on the behavior of the organism, since the scanning beam is of very low intensity and impinges in random fashion on organisms moving in a random way in a drop. Without having to use a dark-field system involving possible directional effects, and without undue constant or intermittent illumination of the organism, one can, by phase adjustment of the flyingspot system, obtain a brilliant track against a dark background on the viewing screen. This of course allows one to expose a small area to an image and obtain a track (Fig. 9A), the length of which depends upon exposure time. The figure shows a series of discrete "blips" which mark the track of a schistosome miracidium moving in a water drop. For this record the scanning rate was 12.5 times per second; hence, in 1 second's travel there were 12.5 discrete blips. With dimensions known and calibrated, it was of course possible to compute the velocity of a single organism with great rapidity from the developed film. The

speed with which the experimenter could record the pathways of a large number of single organisms was limited only by his dexterity in setting up drops for the apparatus. There is, of course, in such apparatus no reason to limit oneself to one organism per drop, just so long as crowding does not introduce extraneous stimuli resulting from random encounter.

These miracidia were shown to travel at approximately 1.8 mm per second. Normally, the path of a miracidium in a drop of water is straight and involves smooth gliding motion more or less directly from meniscus to meniscus across a drop. There is, of course, no reason to assume that under natural conditions such an organism proceeds in a straight line



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FIG. 9. Two-second exposures of the tracks of the miracidium of *Schistosoma mansoni*. A, in a water drop. B, in filtered extract of whole ground host snail, *Australorbis glabratus*. At the right in B the "whirling" activity frequently exhibited on first encountering host extract.

for a long period of time, but nevertheless a rate of linear velocity in excess of 6 meters/hour must be of some ecological significance in the dispersal of the invasive stages of this dangerous parasite.

Unfortunately, it was not possible to carry out quantitative studies on the klinokinetic behavior of this miracidium, although Fig. 10B shows the marked change in the rate of random turning which occurs when the miracidium is placed in extract of whole, ground, host snail. Here again, it becomes absolutely necessary to find out how this response, which appears to be relatively specific for material from the host snail, helps the miracidium to find the snail.

Recently similar apparatus in which the optical system has been reduced to a minimum has been developed for the writer's laboratory (Fig. 10). This is particularly well suited to the recording of pathways of organisms within a wide range of size variation, between about 15 and

100 μ . At the present time the possibilities for the use of this apparatus are being explored in a study of the free-living phytoplankter *Gonyaulax polyhedra* Stein. With it as a test organism, we have made thorough studies of the effects of changes of salinity and temperature on linear velocity and rate of change of direction in the individual cell. In the course of these studies it has also been found possible to obtain very precise data on the effects of light on a strongly photopositive organism. Fortunately, illumination of the moving organisms in the drop by the passage of a strong beam of light through the drop parallel to the plane of the slide, in no way affects the potential changes occurring in the photocell which result from the scattering of the light of the scanning

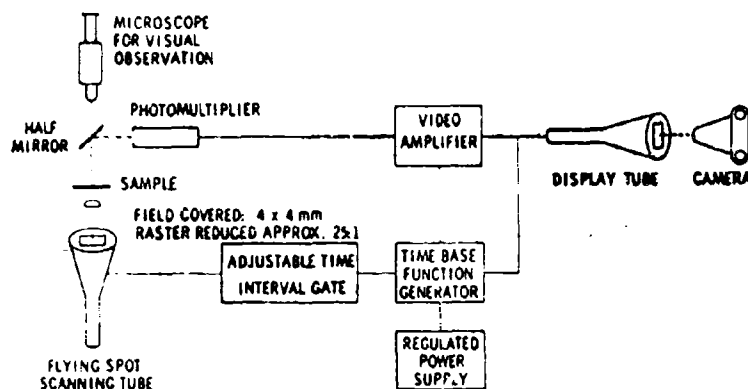


FIG. 10. Block diagram of the scanning apparatus.

beam by the organism. One can therefore record the "normal" pathway of an organism and then record its pathway under directional stimulus from a strong light beam. In strongly photopositive organisms the number of organisms on the viewing screen decreases with time when they are under directional illumination, as a result of their movement toward the light source. One has but to take a series of photographs and record the number of cells in the drop after the passage of a number of fixed time intervals during which the organisms are exposed to the light beam in order to obtain data on this rate of departure. This will be a function of change in linear velocity or rate of change of direction under the illumination. By this technique it will be possible to obtain data on the effects of variation in intensity and wavelength of a directional light stimulus.

To date no further investigations have been conducted with this

apparatus upon adaptive responses in symbiotic organisms. At the time of writing, the apparatus is not engineered to give us information on the behavior of single microorganisms in a chemical gradient. We believe that it should be possible to do this, and if we can, we will return to our analysis of klinokinetic behavior using types of microorganisms in which true taxis behavior involving a directional choice by the organism seems less likely than in an organism with symmetrically arranged, paired sense organs. But perhaps the most significant biological relationship in animate nature involving chemical target-finding is that between egg and sperm. Significant advances in our understanding of the control of movement of sperms have been made by Lord Rothschild (1956) and Brokaw (1958). For the most part, however, these involve the recording of the behavior of numbers of individuals rather than single individuals, although more recently in unpublished work, Brokaw has cast much light on the machinery governing the behavior of individual fern sperms. It is hoped that future modification of the apparatus described above to increase the efficiency of the optical system will make it possible to scan and record the activity of the single sperm cell under varying conditions. The importance of obtaining more quantitative data on linear velocity and rate of change of direction in the individual sperm cell is obvious.

The above has been presented as an indication of how the experimental approach to behavior in symbioses can advance our understanding of fundamental biological phenomena as well as cast light on symbioses per se. This has not only involved such phenomena as the physiology of target-finding, or the nature of chemical signals, or the above-described bioassay of spectra of hosts. Many other types of biological phenomena can be investigated with the use of symbioses, and we may present two types as examples. Fundamental advances in our understanding of the neurophysiology and the behavior of coelenterates and of the control of their so-called independent effectors, the nematocysts, have been made with the use of certain interspecific relationships involving coelenterates. Invertebrate zoologists and students of behavior have become increasingly interested in gaining an understanding of what coelenterates can do. It has long been thought that their nervous system consists of a relatively simple nerve net; as is well known, Professor Carl F. A. Pantin of Cambridge University and his students have investigated coelenterate neurophysiology at great length and set the stage for more recent investigations. Many neurophysiologists feel strongly that a thorough understanding of the function of the coelenterate system can contribute greatly to our understanding of more complex systems of organization. Theoretical coelenterate nerve nets have been created recently and studied on computer systems. Perhaps the most important and indeed

dramatic demonstration of the capabilities of an actinian has recently been made by Ross (1960) of the University of Alberta, who has carried on a series of elegant experiments in the course of investigating the relationship between the European hermit crab *Eupagurus bernhardus* (L.) and the sea anemone *Calliactis parasitica* (Couch) which lives on the shell inhabited by the crab. Previous to Ross's work this partnership had been investigated by Fautot (1910, 1932), Brunelli (1913), and Brock (1927). The crucial experiment conducted by Ross which has such direct bearing on coelenterate physiology and behavior was performed at Plymouth, where *Eupagurus* takes little or no part in the activity which results in the change of position of a *Calliactis* from a glass plate or stone to the shell of its host. The behavior of the anemone is so precise and so interesting that it would be best to quote Ross' words directly in a description of the process:

The attachment of *Calliactis*, which have been removed from shells, to stones, glass plates and other objects, is in no way remarkable. An animal lying on its side, or supported in some way, merely secures a foothold by the edge of the pedal disc, and from this foothold the attachment spreads until the whole surface is adherent. The entire process may take many hours and is accomplished by the combined effects of muscular suction and cementing secretions. Essentially this process is the same as the method employed by *Actinia equina* and *Anemonia sulcata*. Compared to these, however, the pedal disc of *Calliactis* attaches much more slowly, but once the attachment is made it is firmer, and because the attached foot is virtually immobile, it is more permanent.

The attachment of an unattached *Calliactis* to a shell occupied by *E. bernhardus* is very different indeed. Fautot (1910, 1932) described this briefly and the following expanded account, based on my own observations, agrees with his in the essential points. First, the tentacles explore the surface of the shell very actively and many of them adhere to the shell, perhaps by glutinant nematocytes, forming an attachment firm enough to hold the anemone on the shell even when the crab moves about. This tentacular attachment develops within a few minutes into a firmer attachment in which the whole oral disc is involved as well. Apparently, the radial musculature contracts and pulls against the expanded margin and its adhering tentacles and so produces an immense suctorial disc. At no other time does the animal seem so much to deserve its specific name "*parasitica*." Once this is achieved, the anemone is virtually safe from being dislodged, even by the most active movements of the crab.

All this may take 5 to 10 minutes and is followed at once by a slow bending of the column which, in another few minutes, brings the pedal disc up to the shell so that the animal is bent double. The pedal disc, meanwhile, becomes greatly distended and begins to adhere to the shell immediately contact is established. Within another few minutes the whole pedal disc has spread out, its swollen surface and edge becoming bedded down firmly to fit the grooved, often encrusted, surface of the shell. Finally, when the pedal disc is firmly attached, the tentacles and oral disc let go, and in the

space of another 2 to 3 minutes, the column straightens out and the animal assumes its normal extended posture.

Ross was indeed fortunate, because as a result of his extensive earlier experience with coelenterates and with the form *Calliactis* in particular, and because he immediately observed that *Eupagurus bernhardus* in Plymouth waters apparently does nothing to aid the transfer of the anemone to the shell, he was enabled to concentrate his entire attention on the activities of the coelenterate. Only thus was it possible for him to discover what we agree "is surely one of the most remarkable behavior patterns to be found in the normally sessile coelenterate animals." This work in fact has considerably broadened the spectrum of behavioral activities that we know can be performed by actinians, for it clearly involves at least relatively specific recognition, followed by an integrated and coordinated act which results in the anemone taking position on the shell. Ross has shown that it is the periostracum of the host's shell which is the chemical "releaser" for the activity. He has even been able to elicit the attachment of the tentacles, the separation of the pedal disc, and the beginning of the climbing activity by merely bringing in contact with the tentacles a piece of periostracum which has been removed from the shell, dried, and placed in a box for several months! These researches go far to support the well-known investigations of Professor Pantin which have, of course, indicated that sea anemones have a fairly wide spectrum of behavior. But it was not until this behavior of *Calliactis* and that of the anemone *Stomphia coccinea* Müller were discovered, in both of which a sequential series of integrated behavioral acts are performed in a relatively short time, that we had an opportunity to grasp how important specific recognition may be in these animals, or that they may indeed perform highly coordinated behavioral acts with an adaptive end. The work of Yentsch and Pierce (1955), Sund (1958), Hoyle (1960), and Robson (1961a,b) has done much to clarify our understanding of the fascinating physiology and behavior of *Stomphia*. Although in this particular actinian a negative (escape) response to a predator is involved rather than a positive response to a host, the observed phenomena are so close to those observed by Ross in *Calliactis* that they are worth mentioning here. *Stomphia coccinea* has been demonstrated to escape from certain other marine animals. The anemone responds to contact from these animals by lengthening itself, separating its disc from the substrate, and literally swimming away with an active whip-like activity. In the Pacific Northwest, where much of the work on the animal has been done, it has been demonstrated to respond with greatest intensity to a number of starfishes, particularly *Dermasterias imbricata* (Grube) and *Hippasterias spinosa* Verrill. Dr. Robson (1961b), working at the

Danish laboratory in Helsingør, found that the *Stomphia* of north European waters escaped in this manner from the predacious nudibranch *Teolidia papillosa* (L.). It is interesting that the escape response of the Pacific *Stomphia* to certain starfishes occurs at all; there seems to be reasonable doubt that the starfishes concerned occur in the same immediate environment as *Stomphia* and even if they do there appears little likelihood that they would be predacious on these anemones. One may well wonder whether a lock-and-key-type of chemical recognition is involved here in which the lock sensors of the anemone are opened by a key chemical which is fortuitously produced by certain starfishes in no way related to that organism from which the anemone is adaptively organized to escape. Although electrical stimulation will induce the escape response it would certainly appear that in nature the escape response is only elicited by precise chemical recognition. Robson has shown that the receipt of this chemical information triggers the activity of a "pacemaker" or center in the basal region of the anemone. Similarly, Ross has demonstrated the importance of the basal region of *Calliactis* in the information exchange which triggers shell attachment activity, for the tentacles of an animal from which the basal disc has been removed fail to attach to the host shell, while half of an anemone which has been transected along the oral-aboral axis will successfully carry out the entire attachment activity.

We have therefore seen how with these two coelenterates some rather fundamental advances have been made in our understanding of the behavioral physiology of the group; it is clear that both *Calliactis* and *Stomphia* are admirably suited to further investigations in sensory physiology, information transfer, and integrated activity control. But in still another way both organisms are showing themselves to be useful in the investigation of basic phenomena in the biology of coelenterates, for with them some highly interesting information has been obtained concerning the activity of those unique defensive and prey-capturing structures, the nematocysts. To understand how it became possible to establish the controllability of nematocysts by certain coelenterates, some earlier experiments must be described. From the beginning of his interest in symbiosis, the writer had been fascinated with the symbiosis between certain giant sea anemones (*Stoichartis* sp.) and a number of genera of pomacentrid fishes, particularly the genera *Amphiprion* and *Premnas* (Fig. 3). Significant observations on this symbiosis had been made by Sluiter (1888), Verwey (1930), and Gohar (1948), who had given us some knowledge of the ecology and behavioral characteristics of the animals in the partnership. In 1947 Gudger reviewed all the observations that had been made up to that time, and in 1950 Baerends first speculated

about the possible role of releasers in the maintenance of the association. In 1958 the author had the opportunity to work with Dr. Kenneth S. Norris on the partnership between the giant anemone and *Amphiprion percula* (Lacépède). A single giant anemone was available for experimentation. About a dozen *Amphiprion percula* were used in the experiments which had been kept separated from their host for several months. With these animals it was possible to demonstrate that there is a rapidly acting, specific, heat-labile factor present in the mucus on the surface of the fish which markedly raises the threshold of discharge of the host's nematocysts. Fishes which do not possess this factor (i.e., other species) are immediately stung and eaten. *Amphiprion* are immediately stung and consumed by anemones such as *Anthopleura* against the nematocysts of which the mucus of *Amphiprion* is ineffective. The experiments to establish these facts were at the time, of course, fascinating to perform, but the results were perhaps to be expected. They cast very little light on a problem which is of the greatest interest to the student of invertebrate behavior, namely, "What can a coelenterate do?" Yet observations on the behavior of the fish may very possibly have given the first insight into possibilities in the coelenterates up to now not foreseen. It has long ago been shown that a coelenterate will respond in specific ways to certain stimuli coming from the external environment, but it has never been clearly demonstrated that a coelenterate can store information and modify its behavior in accordance with certain information previously received. The writer and Dr. Norris repeatedly observed a very particular type of behavior on the part of *Amphiprion percula* during what we called the "process of acclimation." Very little indeed is known about how frequently this process occurs in nature, how much of the adult life of these inquiline fishes is spent in a free state or how much they move from host to host. But when the individual "unacclimated" *Amphiprion percula* was introduced into the observation tank with the anemone, a fairly stereotyped series of events occurred which terminated in acclimation. To quote from Davenport and Norris (1958):

An unacclimated fish introduced into the tank a foot or so away from the anemone usually approached the anemone within a few minutes and began to swim under the disk around the column, and occasionally over the top of the disk a centimeter or more away from the tentacles. Such fish spent most of their time under the disk at this stage and sometimes were seen nibbling at the column of the anemone. Most fish seemed to "recognize" the anemone within a few minutes and swam toward it. . . . As the process proceeded, passage over the disk became more and more frequent and the "acclimating" fish moved closer and closer to the tentacles. Swimming was accomplished by a distinctive series of slow vertical undulations, in which the tail was usually held a little lower than the rest of the body. Eventually,

on one of these trips over the disk, the fish would touch a tentacle or two, usually with the ventral edge of its anal fin or the lower margin of its caudal fin. Commonly this resulted in a moderate adherence of the tentacle to the fin and contraction of the tentacle. The fish then jerked itself free with a violent flexure of its body and usually raced off the disk. Not all newly introduced *Amphiprion* caused clinging upon their first contact with tentacles, but it was the general rule. However, this adherence failed to deter the fish, which nearly always returned immediately to the anemone, either under the disk or over the tentacles . . .

After this initial contact the fish typically came closer and closer to the tentacles, touching them with increasing regularity. The reaction to the clinging of tentacles became less and less violent until a sudden flexure of the animal's body was the only reaction given by the fish. Mouthing or nipping of tentacles was often observed in this and later stages.

The clinging and contraction of tentacles upon contact with the fish gradually became less until it ceased altogether. At the same time the fish began to swim deeper among the tentacles, using the same slow undulating movements as when it had cruised above the disk.

Once the fish was swimming in fairly constant contact with the tentacles of the anemone, a very striking change in its behavior occurred. The general speed of swimming suddenly increased until the *Amphiprion* was dashing back and forth over the disk of the anemone, flailing unreactive tentacles aside with violent movements of its body. Often the fish raced beneath the anemone and appeared in one of the folds of the disk margin, its head completely ringed in tentacles. The fish frequently maintained this vantage point for a few seconds, holding position with rapid alternate fanning movements of its pectoral fins, after which it might dash onto the disk again for another foray among the tentacles. The powerful swimming typical of this stage of the acclimation process was accomplished by rapid and strong lateral body flexures. The impression given by the swimming behavior of the fish after final acclimation was that the fish was "bathing" its entire surface among the tentacles.

This final activity was so marked, so sudden, and so dramatic that upon observing it one was obliged to recall the rejoinder of Chuang-tzu to Hui-tzu quoted above, for once an *Amphiprion* reached this level in its behavior, it exhibited every indication of "joyful" activity in its mad dash in and out of the tentacles of its host. The point to be made is that the activity of the free-living fish, which lives perfectly successfully for months in tropical aquaria and is kept thus by numerous tropical fish establishments, is entirely different than its behavior when it is in the anemone. The behavior in the anemone is specialized. What function does it perform? There are two possibilities. The first is that the activity does something to maintain the protection which the mucus gives to the fish. It may well be that continued active contact with the tentacles induces mucus production, which in turn strengthens the protection of the fish against the nematocysts. But it is obvious that there is at least

one-directional information exchange between the two animals, for without question the fish receives stimuli from the anemone which elicit this specialized sort of swimming behavior. Yet some of the fishes used in these experiments appeared to need no lasting contact with the anemone to get full protection against nematocyst discharge; they entered the anemone immediately without receiving any adverse stimuli. We are beginning to believe that a second possibility is that this activity of the fish may be directed to causing the anemone to "recognize" it and to withhold nematocyst discharge. In spite of the fact that it is generally supposed that nematocysts are not under nervous control but that they fire off independently upon adequate stimulation, several investigators have speculated that in such partnerships the presence of the fish in some way causes the coelenterate host to put its nematocysts "out of action" (Baerends, 1957). This possibility seems all the more likely now that it has been discovered that in certain anemones the receipt of specific chemical signals at a point on the animal remote from the tentacles causes information transfer to the tentacles which inhibits nematocyst discharge.

In 1960, the writer was working at the Laboratoire Arago at Banyuls at a time when Ross and Sutton (1961a,b) were investigating the above-described fascinating behavior of *Calliactis parasitica* in its recognition of the shell of its host hermit crab. Ross had noted that when a *Calliactis* had settled on a glass disc its tentacles were sticky to the touch, but when it was already on the host's shell there was no such stickiness. Experiments which followed (Davenport, *et al.*, 1961) indicated conclusively that the nematocysts of *Calliactis* at least can by no means be considered independent effectors. Ross (*loc. cit.*) had shown that one of the most important steps in the behavioral sequence involved in the series of events occurring when *Calliactis* climbs on its host's shell is the exploration of the surface of the shell by the tentacles of the anemone and the adherence to the shell of many of the tentacles. Ross had guessed that this adherence was "perhaps by glutinant nematocysts." It was shown in a simple series of experiments in which single tentacles of anemones were touched and scored for adherence or absence of adherence that when *Calliactis* is not *in situ* on its host's shell, the great majority of the tentacles respond to contact with shell by immediate adherence, whereas the tentacles of a *Calliactis* which is *in situ* on the host's shell give little or no adherence to shell. The adaptive significance of this phenomenon is of course obvious; were the nematocysts of the tentacles sensitive to shell when the animal is *in situ*, one would have multi-individual chains of symbioses sticking together en masse whenever groups of hermit crabs with *Calliactis* on their shells got together. But the true importance of the phenomenon lies in the fact that it is the reception of specific information

by receptors in the pedal disc of the anemone that results in the inhibition of special shell-sensitive nematocysts in the tentacles. These nematocysts have not been identified. That they are not the same as those released by prey is obvious, for if those nematocysts sensitive to prey were inhibited by this information while the animal was *in situ*, the symbiosis could not exist. For this particular phenomenon in coelenterate biology to be discovered it was necessary to use forms in which information transfer was relatively swift; as is so often true in coelenterate physiology, the time parameter was all important. As yet, it has not been possible to determine the time necessary in *Calliactis* for the transfer of that information from pedal disc to tentacles which results in the inhibition of shell-sensitive nematocysts. But more recently, Ross and Sutton (1964) have shown that in some forms the time for such information transfer may be extremely short; working with *Stomphia* they have demonstrated that those nematocysts of *Stomphia* which are sensitive to the starfish that elicits the swimming response are rapidly inhibited by the onset of swimming activity. This information transfer operates in terms of seconds. There seems every reason to believe that in this case as well as in *Calliactis* the inhibition is the result of nervous activity, for inhibition by hormonal activity would be too slow.

We have seen that the precise recognition of specific chemical agents may be very much a part of the normal behavioral biology of coelenterates. Not only that, but we have also observed that this recognition may result in some highly unique adaptive behavior. Is it not therefore possible that in the widely known partnerships between giant anemones and their inquiline fishes, the activity of the fish may in some way be directed to effecting recognition of the fish by the anemone? It is clear that there are many phenomena under the sun in symbioses involving coelenterates and their partners which at this time are extremely difficult to explain. The writer spent a solid month at the Gatty Laboratory of St. Andrews University in attempting to find out how the symbiotic crab *Hyas araneus* (L.) attains its protection from the large sea anemone *Tealia felina* (L.). If a large *Tealia* is expanded in an aquarium with but a centimeter of water covering the tentacles, one may drop a *Hyas* directly onto the disc of the anemone without eliciting any response whatsoever from it. If one drops a specimen of the common shore crab *Carcinus maenas* (L.) onto the *Tealia*, it is engulfed in a flash. An attempt was made to discern the method whereby the behavior of the anemone is controlled under these circumstances. As in the case of the anemone-fish partnership the anemone exhibits no overt, easily recognizable response to its partner as does, for instance, *Calliactis* to the shell of its partner pagurid. The only clearly discernible phenomenon resulting from the

introduction of the crab is the *failure* of the anemone to respond as it would to other crabs. In the case of the anemone-fish partnership one suspects that the inhibition of nematocyst discharge by the mucus of the fish may effectively prevent muscular response by the anemone; perhaps nematocyst discharge triggers the feeding response of the anemone. But a *Hyas* dropped on a *Tealia* causes great nematocyst discharge. The *Hyas* generally sticks rather firmly to the tentacles and can only move about with difficulty, walking like a man in hot tar, yet ordinarily there is no closure response from the anemone and no infolding of the disc. Everything possible was tried, such as dipping a "masked" *Hyas* into *Carcinus* body fluids (which when absorbed on a sponge normally cause immediate closure). When such an animal was dropped, *nothing* happened. The anemone simply "recognized" its partner and no sign of a closure response occurred. It is obvious that we must learn a great deal more about the abilities of coelenterates before we can clearly understand the machinery controlling symbioses involving them.

VIII. The Role of "Learning"

We must now turn our attention to the problem of the role of learning and associated phenomena in symbioses. Twenty-two centuries ago the Taoist said: "Creatures with nine channels of communication are born from the womb. Creatures with eight are born from the egg." The proportionality of complexity with ability to exchange (and, indeed, store) information has been understood for a very long time. Obviously, the extent to which these phenomena are important in symbioses is directly proportional to the physiological abilities of the major taxonomic groups to which partners belong. It emerges that the information-storage abilities of lower phyla have in recent years been demonstrated to be considerably greater than had previously been suspected. As early as 1916, Brancelli, as Thorpe (1963) says, "was forced to assume an elaborate instinctive organization together with an ability for true associative memory" in actinians which climb onto their hosts Crustacea. Such assumptions for the information-storage capacity of as simply organized creatures as actinians have to a great extent been rejected by zoologists since the turn of the century. However, as we have seen, Thorpe is quite correct in saying "the matter should well repay critical study and experiment in the light of more recent knowledge."

The various types of information storage have been categorized in a number of ways, i.e., "habituation," "associative learning," "latent learning," "insight learning," etc. We may assume that of this spectrum of learning categories the fewest categories are exhibited in the simplest

animals. Yet, as is made abundantly clear by Thorpe, the assumption that the higher categories of learning cannot be found among invertebrate phyla must be abandoned. He says: "The neglect of the study of invertebrate behavior has given the impression that insight-learning" (which he defines as the sudden production of a new adaptive response not arrived at by trial behavior) "is a characteristic human faculty hardly to be expected in a subprimate mammal and, of course, out of the question in an arthropod. We now see what an astonishing misconception this is." Taking our association Man-Dog as a standard, we see that both partners in the association exhibit a wide spectrum of learning abilities. This symbiosis is almost as complicated from the behavioral point of view as any which exists, for just this reason. What is the situation in the simpler symbioses we have so far considered? At present, we are in the midst of a "renaissance" of investigation, largely neglected since Jennings' day, of the role of learning and associated phenomena in invertebrates (Thorpe and Davenport, 1965); however the precise role of learning in symbiotic partners has so far only been obliquely approached. A number of highly interesting experiments have been conducted which in varying ways bring light to bear upon the manner in which information storage may be of importance to the maintenance of symbioses. Habituation may be defined "as the relatively permanent waning of a response as a result of a repeated stimulation which is not followed by any kind of reinforcement. It is specific to the stimulus and relatively enduring and so it is natural to regard it, provisionally at any rate as distinct from fatigue and sensory adaptation" (Thorpe, *loc. cit.*). There are no controlled experiments which demonstrate the fact, but there is every reason to assume that this type of information storage may be of importance, particularly in those phyla in which certain associated animals maintain their protection against the predacious activities of hosts. We have already hypothesized that the particular swimming activity of the commensal fishes living in giant sea anemones may be directed to the habituation of the hosts to specific stimuli coming from the fish. Blösch (1961) takes some issue with Davenport and Norris (1958) about the role of the protective mucus coat of *Amphiprion* in protecting it against the feeding activities of its host. In his paper he gives no experimental data and describes no controls; in particular, he does not mention his method of handling fishes, which is very critical in such experiments. However, he comes to the highly intriguing conclusion that giant anemones which have been isolated from their commensal species always sting these fishes on first contact *even if the fishes have been taken directly from other host individuals*. If this phenomenon can be firmly established, it would seem reasonable to assume that associative learning may also be of importance in coelenter-

ates. If it is true that a fish taken from an anemone and reintroduced to it is *not* stung, while a fish taken from another anemone of the same species *is* stung by the first host, one might assume that the anemone recognizes its own fish as a result of the association of certain stimuli received from it as an individual. In this case some particular reinforcing stimuli may be involved.

We should therefore be able to find out a good deal more about the information-storage capacities of coelenterates by using symbiotic or prey-predator relationships involving these animals. Complex integrated behavior occurs when a center or pacemaker is stimulated by specific stimuli coming from host (or predator) contact in *Calliactis* and *Stomphia*. It should be possible to combine the specific stimulus eliciting this activity with another stimulus and perhaps ultimately replace the first with the second. No one has tried this yet. Someone should, for such an experiment might establish once and for all the existence of associative learning in Coelenterata.

Associative learning has been demonstrated beyond a reasonable doubt in flatworms and annelids. The researches of McConnell and his colleagues (1959, 1960) in which it has been shown not only that conditioning takes place *throughout* the soma of a flatworm rather than just in the anterior ganglia, and also that such conditioning may be transmitted when a trained flatworm is ingested by an untrained flatworm, have changed much of our thinking about the significance of learning in the lower invertebrate phyla. Jacobson (1963) has recently published an able review of the role of learning in flatworms and annelids. To date there has been no experimental work directed to the role of learning in the control of symbioses involving these animals, but one can hardly doubt in view of the above experiments that in symbiotic species some coding must exist which helps to "cement" partnerships. The importance of separating the roles of genetically determined coding and coding resulting from experience ("olfactory conditioning," imprinting, higher learning categories) can be observed when we consider the life histories of certain associated animals which are "active seekers." Environmental coding may be superimposed upon a genetic coding which involves, at least in those animals that have free-living larvae, a precise response to the host which triggers metamorphosis. The writer is aware of no publications to date in which the signal from a host eliciting the metamorphosis of a settling associate has been identified. The genetically determined physiological abilities controlling settlement and metamorphosis in associated invertebrates could be investigated in almost any readily available partnership. Those factors which tend to bring the larvae to the vicinity of the host would have to be investigated; as Laing (1937) said in

her interesting studies of the behavior of the chalcid fly *Alysia*: "... some parasites find environments first and hosts later. . . . They may often use quite different senses for the perception of the two and make quite different movements to reach them. What these senses and movements are however, will differ greatly with different parasites, and must be especially determined in each particular case."

Next, the factors eliciting specific settlement and metamorphosis must be elucidated. Without question, the machinery will range from fairly simple phenomena exhibited by larvae which as a result of certain coded responses to light, gravity, etc., are brought by winds or currents to the vicinity of their hosts where a precise recognition occurs which effects settlement, to complicated behavior such as that which must be present in such forms as the parasitic copepod *Athelges* (Thorson, personal communication). In this species which parasitizes pagurids in north European seas, males are never found alone on the host. About 5% of the hermit crabs of the genus *Anapagurus* have parasitic female *Athelges* attached to their abdomen; without exception these female *Athelges* have small males clinging to them. Several questions immediately arise upon the contemplation of this situation. Are the larvae which are attracted to the hermit crabs sexually undetermined? If this is the case we must assume that all larvae are attracted to the host and that direct parasitic connection with an unparasitized host directs the development of the parasite to femaleness. A larva subsequently attracted to the host must rapidly fall under the influence of the already attached female of its own species and its development thus directed to maleness. At best, if the larvae are already genetically determined, male larvae must be far more strongly attracted to the females *in situ* on hosts than to parasiteless hosts; indeed, it would seem that if there are male larvae, they may not be attracted to parasiteless hosts at all.

The writer is aware of no experiments that have demonstrated that behavioral differences observable between free-living and associated populations of single species are genetically determined. Unfortunately, he was unable to complete experiments designed to compare the difference in behavior of larvae resulting from an F_1 cross between a free-living and an associated *Podarke pugettensis*, the polychaete worm which may be associated with the starfish *Patiria miniata*. Experiments of this sort must be carried out successfully if we are to understand the role of genetic determination of settlement in associated forms and separate from them phenomena which are dependent upon associative learning.

It seems reasonable to assume as a result of certain highly interesting experiments that one type of information storage occurring during development (olfactory conditioning) may be of special importance in the

maintenance of symbiotic partnerships. While considering these experiments it should be kept in mind that although they involved chemical factors, there is no reason whatever to assume that the effects of other environmental stimuli might not be similarly coded during development. In 1937 Thorpe and Jones found that if one artificially introduced the eggs of the parasitic wasp *Nemeritis*, normally a parasite of the moth *Ephestia*, into the abnormal host wax moth *Meliphora* (to which *Nemeritis* normally gives no response) the resulting adults would demonstrate a strong response in an olfactometer to the abnormal host when they were faced with a choice between *Meliphora* and a blank. These workers were never able to demonstrate in such conditioned adult wasps an egg-laying preference for the abnormal host over *Ephestia*. More important, perhaps, Thorpe (1939) later demonstrated that one could elicit a strong response from adult *Drosophila* to a number of chemical agents which had been introduced into the environment in which they had been raised. The crucial point here is that the animal undergoes development *continuously* under the influence of certain stimuli, and that this information is in some way stored so that the behavior of the adult may be affected by these same stimuli. There is no great distinction between this phenomenon and that known as imprinting (Ramsay and Hess, 1954), although, by definition, for imprinting to occur the developing organism must be subject to the specifically acting stimuli during a *critical time period* in its development. Finally, the experiments of McConnell have demonstrated that in animals of the most simple acoelomate, triploblastic structure information may be coded during life throughout the soma. These and such researches as the elegant experiments of Horridge (1962), in which a single isolated thoracic segment of an insect has been trained to hold its leg up out of a pool of mercury, have made it clear that we must direct much more of our attention to the problem of the role of learning in the maintenance of symbioses, particularly in symbioses involving members of those phyla the learning abilities of which have previously been unsuspected.

It would seem fitting to end this volume with a tribute. From the beginning to the end of his classic "Le Parasitisme et la Symbiose," Maurice Caullery stressed the importance of the experimental approach. All who have worked with animal associations owe a debt to the great French zoologist. We can hardly do better than to quote his own words of conclusion, as timely today as when they were written:

"Les associations réalisées sous les diverses formes du commensalisme, du parasitisme et de la symbiose entraînent, chez les organismes participants, des activités fonctionnelles et des réalisations spéciales, que l'on ne constate pas chez les formes libres. De là de multiples problèmes d'ordre non seule-

ment morphologique, mais aussi et peut-être surtout physiologique, problèmes extrêmement complexes, que nous commençons seulement à entrevoir et à aborder, et dont l'intérêt ne saurait être surestimé. Sous des aspects que se transformeront plus ou moins, l'objet qui forme la base de cet ouvrage restera longtemps à l'ordre du jour de la Biologie."

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